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LIQUID CHROMATOGRAPHIC ANALYSIS OF THE FREE SUGARS IN SWEET CORN: A METHOD INDICATIVE OF MATURITY AND OF QUALITY CHANGES RELATED TO PROCESSING TECHNIQUES

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Food Sciences Laboratory

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The utility of high performance liquid chromatography for the rapid analysis of individual free sugars in foods is demonstrated. The sucrose content of sweet corn (var. Jubilee) was shown to depend on the maturity of the corn when harvested. A maximum value was obtained in corn picked 25 days after 'half-silk'. Changes in the sugar content due to subsequent processing (i.e., blanching and sulfite treatment, freeze-drying and compression) were of the order anticipated. Moisture content and alcohol-insoluble solids are also reported for all samples.		

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PREFACE

There is a continuing need for objective methodology which can aid in the assessment of food quality. Two criteria of quality, which are amenable to analytical chemical techniques, are those of nutrition and flavor, and the free sugar content of foods is important in both of these areas. This report describes the use of high pressure liquid chromatography to determine the free sugars in samples of sweet corn (variety Jubilee), which had been harvested at five different stages of maturity. The effects on sugar content due to additional blanching and sulfiting and to freeze-drying and compression are also examined.

The corn used for the analysis was grown at the New York State Agricultural Experiment Station, Cornell University under Contract No. DAAG17-75-M-0684 with the Plant Products Group, Food Engineering Laboratory, with Dr. Abdul R. Rahman acting as Project Officer.

We would like to thank Dr. Rahman, Head, Research and Development, Plant Products Group, for his cooperation and support of this work. Funding was provided under projects 1L762724AH99, "Food Processing and Preservation Techniques" and 1Y161102AH52, "Food Analysis and Composition".

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LIQUID CHROMATOGRAPHIC ANALYSIS OF THE FREE SUGARS IN SWEET CORN:

A Method Indicative of Maturity and of Quality Changes Related to Processing Techniques

INTRODUCTION

For sweet corn (Zea mays saccharata), the sugar content is one of the primary criteria of flavor. Multiple regression analysis of sensory panel scores indicated that sweetness and texture were most important in overall flavor and that aroma played a relatively small part.[1] The relative importance of sweetness and texture were about equal for fresh corn, but for processed corn (canned or frozen), sweetness showed the highest positive correlation with flavor. More specific information was obtained in a separate investigation, in which it was found that the highest rating of corn on the hedonic scale correlated with the highest sucrose concentration and not at all with the concentrations of glucose and fructose.[2] A rapid analytical method for the determination of sucrose should, therefore, provide a convenient and objective indicator of the flavor of corn.

Until recently, methods for the analysis of individual sugars have been time-consuming, and therefore, expensive. Paper chromatographic methods[3,4,5] are tedious and involve multistep procedures. Methods utilizing ion-exchange resins,[6,7,8] although automated, are still complex and time-consuming. Gas liquid chromatography (g.l.c.) has found considerable application in the

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[1] L. F. Flora and R. C. Wiley, J. Food Sci., 39, 770 (1974).

[2] G. Rumpf, J. Mawson and H. Hansen, J. Sci. Food Agric., 23, 193 (1972).

[3] L Hough and J. K. N. Jones, Methods in Carbohydrate Chemistry (R. L. Whistler and M. L. Wolfrom, eds.), Vol. I., Academic Press, New York, 1962, pp. 21-31.

[4] R. L. Whistler and J. N. BeMiller, Methods in Carbohydrate Chemistry (R. L. Whistler and M. L. Wolfrom, eds.), Vol. I., Academic Press, New York, 1962, pp. 395-399.

[5] B. G. Chan and J. C. Cain, J. Chromatog., 22, 95 (1966).

[6] E. Martinsson and O. Samuelson, J. Chromatog., 50, 429 (1970).

[7] L. Hough, J. V. S. Jones and P. Wusteman, Carbohydr. Res., 21, 9 (1972).

[8] J. S. Hobbs and J. G. Lawrence, J. Chromatog., 72, 311 (1972).

analysis of free sugars.[9,10] This technique affords high resolution, but suffers from the inherent disadvantages that volatile derivatives must be prepared and that reducing sugars give multiple peaks. Both of these factors complicate quantitative measurements, but reasonably accurate results are possible.[11,12]

During the last few years, developments in high pressure (performance) liquid chromatography (h.p.l.c.), both in instrument design and column technology, have led to a major advance in sugar analysis. This method, described first by Palmer,[13] permits rapid analysis of aqueous solutions of sugars without derivatization or pretreatment. Small amounts (20 μg) of individual sugars can be quantitatively determined with good precision, and all sugars give single peaks (due presumably to rapid equilibration of anomers on the column). Interference by salts and other noncarbohydrate materials has not been observed, although, where feasible, solutions are de-ionized prior to chromatography to prolong column lifetime.

MATERIALS AND METHODS

Corn Samples and Extraction Procedures

The sweet corn (variety Jubilee) was grown under contract (DAAG17-75-M-0684) at the New York State Agricultural Experiment Station Cornell University. Five samples of different maturity (M1-M5) were obtained by harvesting at two-day intervals. The first sample (M1) was harvested 76 days after planting, 21 days after "half-silk". The corn was processed (blanched 8-15 min., cut and frozen) on the day following harvesting. It was shipped by refrigerated truck to Natick on 17 November 1975.

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[9] C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, J. Amer. Chem. Soc., 85, 2497 (1963).

[10] J. R. Clamp, T. Bhatti and R. E. Chambers, Methods Biochem. Anal., 19, 229 (1971).

[11] J. M. Richey, H. G. Richey, Jr. and R. Schraer, Analyt. Biochem., 9, 272 (1964).

[12] P. K. Davison and R. Young, J. Chromatog., 41, 12 (1969).

[13] J. K. Palmer, Analyt. Letters, 8, 215 (1975).

Twenty samples for analysis were supplied by the Plant Products Group, Food Engineering Laboratory:

a Frozen corn as received, M1-M5.

b Additionally blanched and sulfited, M1-M5.

c Freeze-dried, M1-M5.

d Freeze-dried and compressed, M1-M5.

Samples received frozen (series a and b) were removed from the plastic bags and allowed to thaw until separable. The kernels were lightly blotted to remove excess surface moisture and a representative sample (20 g) was weighed accurately and homogenized in a Virtis blender with absolute ethanol (40 ml) The homogenate was transferred quantitatively to an Erlenmeyer flask, using an additional 30 ml ethanol for rinsing*, and the suspension was heated for one hour on a steam bath with occasional shaking. The mixture was allowed to cool and then transferred quantitatively to a 100 ml volumetric flask using 80% ethanol for rinsing and to adjust the volume. For the freeze-dried samples (series c and d), a weighed amount (5.4 g) was homogenized with 80% ethanol (40 ml), transferred to an Erlenmeyer flask using 80% ethanol for rinsing, and subsequently treated as for the frozen samples. Prior to chromatography, small amounts (1 to 2 ml) of the extracts were de-ionized by passage through a short column of mixed ion-exchange resins; Dowex AG-3 (OH^-), and AG-50 x 12 (H^+), both 200-400 mesh. (After washing the column with three bed volumes of extract, it was shown that the concentrations of sugars in the effluent were identical with those in the non-de-ionized extract.)

Liquid Chromatography, Equipment and Procedures

Samples were analyzed using a Waters Associates ALC-100 Liquid Chromatograph, equipped with a Model 6000 Solvent Delivery System; differential refractometer detector, sensitivity 1 x 10^{-7} refractive index units; Model U6K Universal Injector; "μ Bondapak/Carbohydrate", 30 cm x 4 mm I.D. stainless steel column. Chromatography was performed at ambient temperature (ca 25°C) using water-acetonitrile mixtures usually 15:85, filtered through a 0.22 μ Millipore filter before use; flow rate 2 to 3 ml/min: sample size 100 μℓ.

Quantitative results were obtained initially using a Texas Instruments Servoriter II recorder (10 mv full-scale, variable chart speed). Standard curves - peak height/concentration - were determined for glucose, fructose, and sucrose These results were later confirmed using a Hewlett Packard Reporting Integrator, Model 3380A.

*With the water content of the corn, this affords a solution which is approximately 80% ethanol.

RESULTS AND DISCUSSION

A Typical chromatogram and printout is shown in Figure 1. The peaks with retention times of 5.83, 6.84, and 10.77 are due, respectively, to fructose, glucose, and sucrose and the amounts are concentrations in g/100 ml.

The variations of free sugar concentration (as % fresh weight or % freeze-dried weight) with maturity for the four different series are shown in Figure 2-5.

A number of features are evident:

a. The sucrose concentration varies consistently in all series, reaching a maximum value at M-3 or M-4.

b. The fructose and glucose concentrations do not vary significantly, although a slight downward trend can be observed.

c. Comparison of Figure 3 with Figure 2 shows as expected that additional blanching and sulfiting results in lower concentrations of all sugars.

d. The results obtained with the freeze-dried corn series and the freeze-dried compressed corn series (Figures 4 and 5) show that this processing has little effect on sugar concentrations.

CONCLUSION

These results illustrate the capability of high pressure liquid chromatography for the rapid and accurate analysis of free sugars in foods, but a few comments on sample treatment prior to analysis are appropriate.

Quantitative data available in the literature show a depletion of 40-60% of the sucrose originally present in sweet corn after 24 hours storage at 25°C.[14,15,16] It must be noted that the samples of corn supplied by the New York Agricultural Experiment Station were each processed on the day following harvest. Loss of sucrose undoubtedly occurred between harvesting and processing and the amount lost may not have been the same for each sample.

In the data supplied with the samples, it is also reported that the blanching times varied from 8 to 15 minutes. Since, as shown in this report, blanching leads to loss of sugar, a constant blanch time should be established to eliminate this variable in subsequent investigations.

C. O. Appleman and J. M. Arthur, J. Agr. Res., 17, 137 (1919).

C. W. Culpepper and C. A. Magoon, J. Agr. Res., 28, 403 (1924).

D. M. Doty, G. M. Smith, J. R. Roach and J. T. Sullivan, Indiana Agr. Exp. Sta. Bull., 503, 31 (1945).

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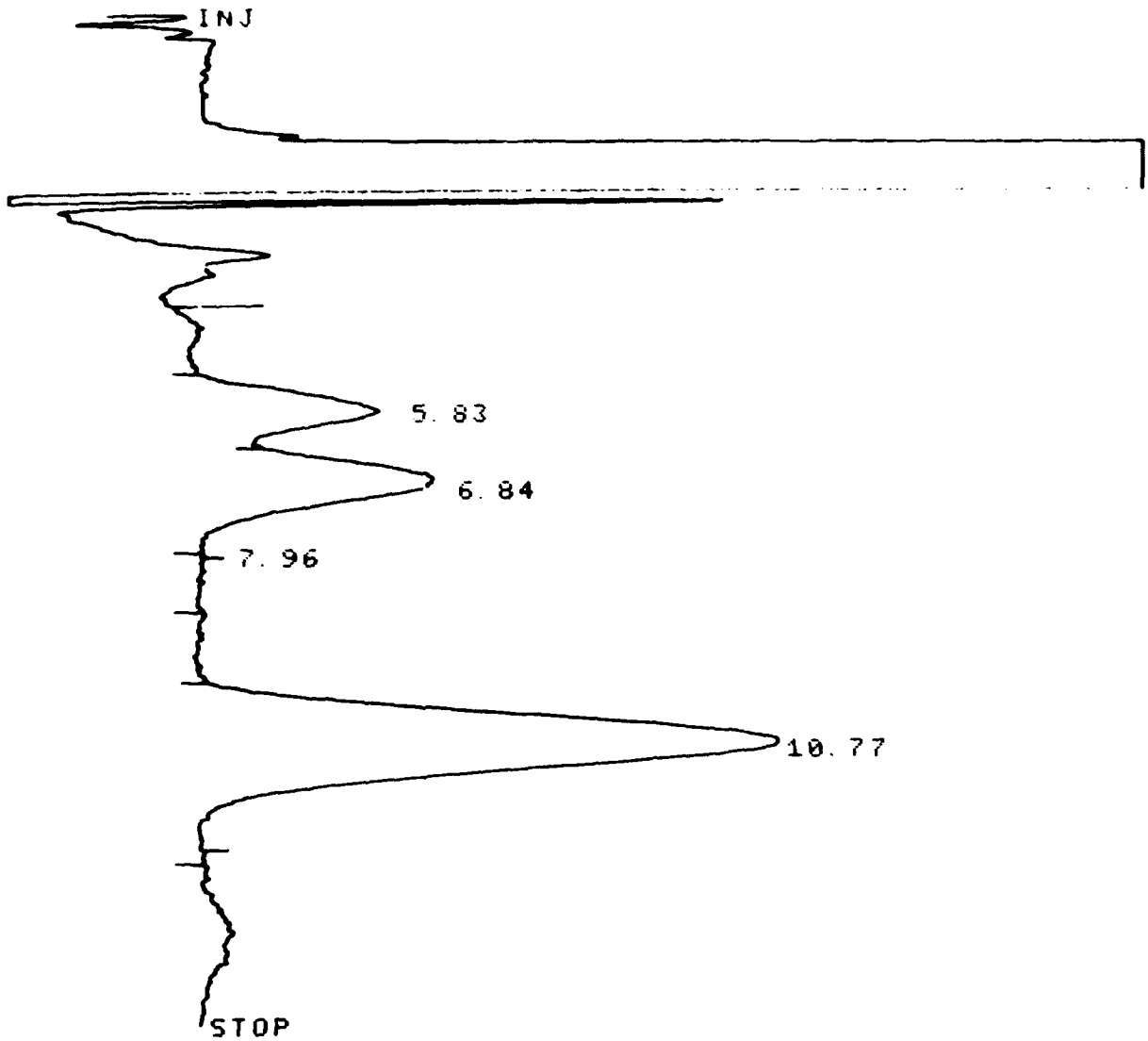
Whistler, R. L. and J. N. BeMiller, Methods in Carbohydrate Chemistry (R. L. Whistler and M. L. Wolfrom, eds.), Vol. I., Academic Press, New York, 1962, pp. 395-399.

FIGURE 1. Chromatogram and Printout for a Typical Corn Extract (Freeze-dried, M-2)



			ESTD NO REF	
RT	TYPE	AREA	ID#	AMT
5.83		193334	4	.049 88
6.84	M	327611	2	.078 59
10.77		955687	3	.221 3
			TOTAL	.349 77

XF 1.

HP 3380A
DLY 4. STOP 15 REJECT 10000
MV/M 3.00 ATTN 32

FIGURE 2. VARIATION OF FREE SUGARS WITH MATURITY

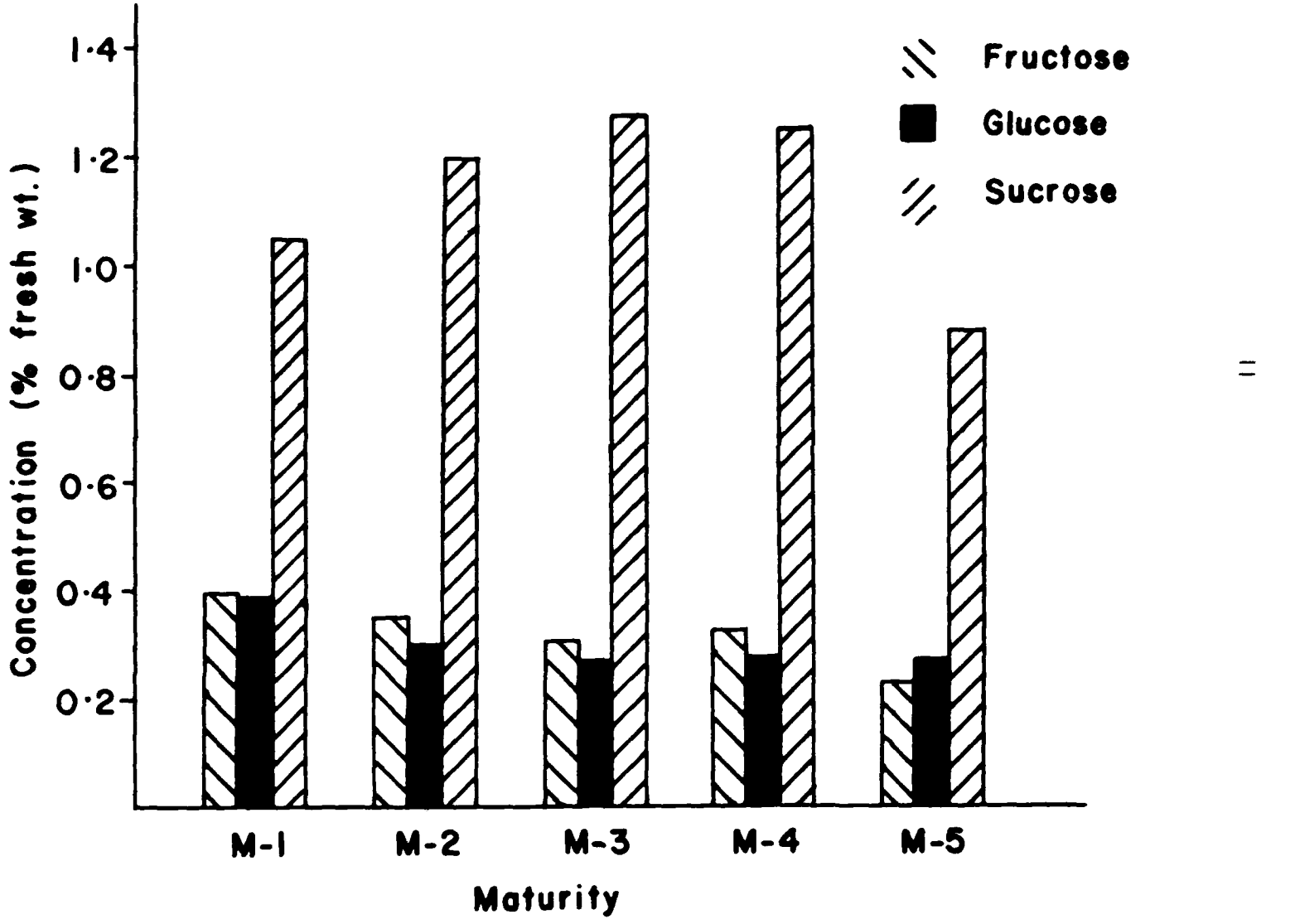


FIGURE 3. VARIATION OF FREE SUGARS WITH MATURITY

b) steam blanched, sulfited and frozen corn

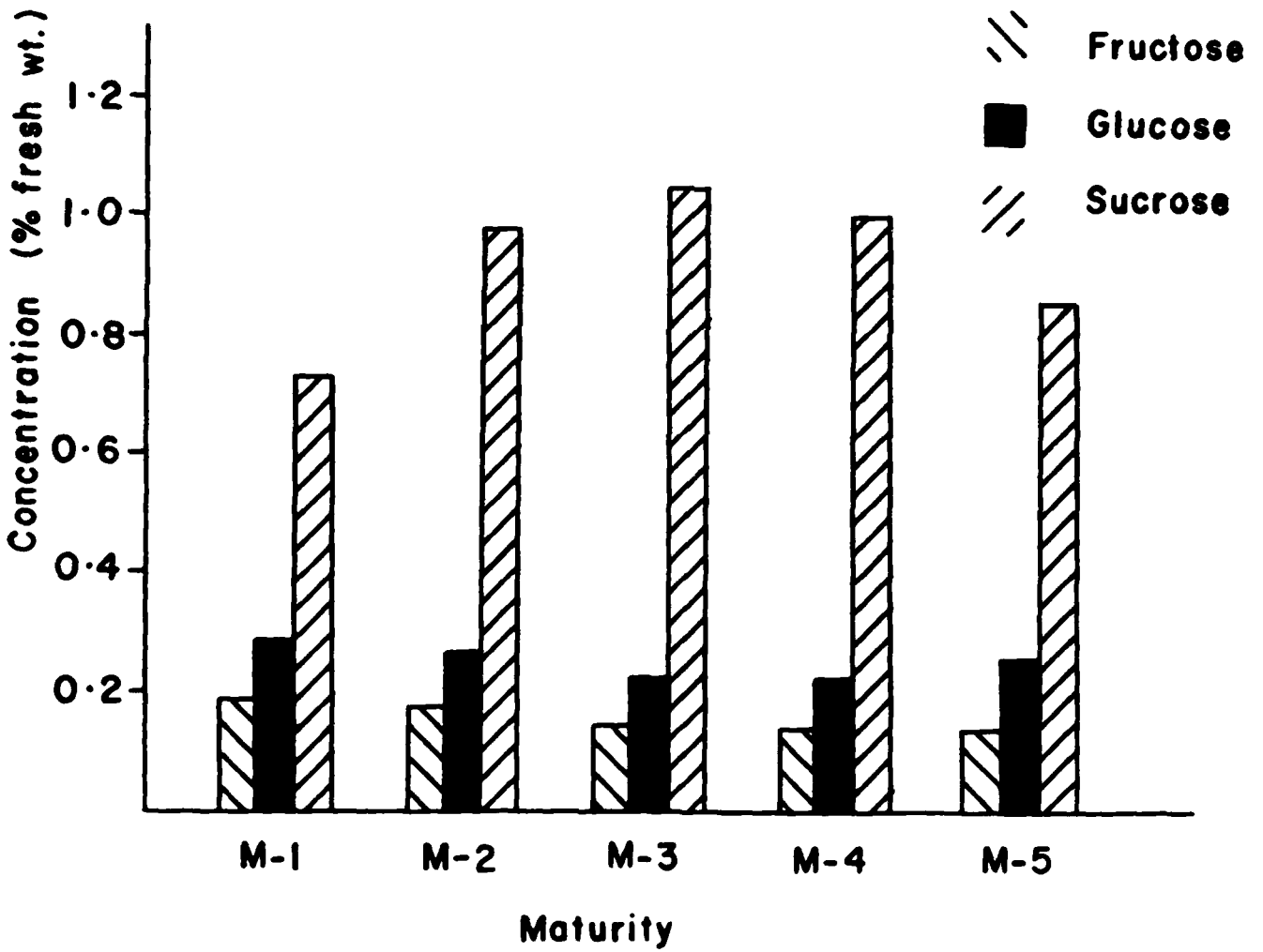


FIGURE 4. VARIATION OF FREE SUGARS WITH MATURITY

c) freeze-dried corn

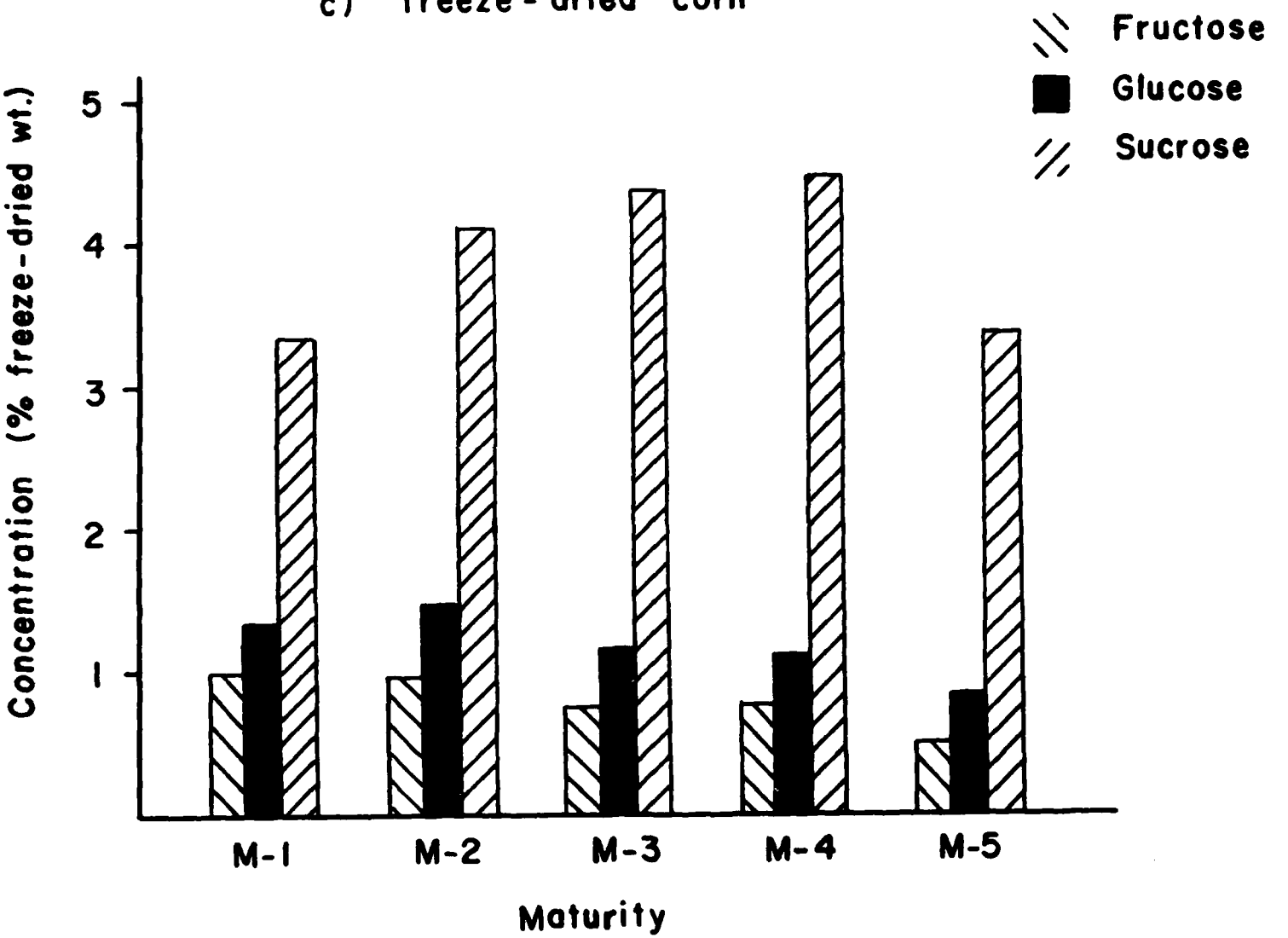


FIGURE 5. VARIATION OF FREE SUGARS WITH MATURITY

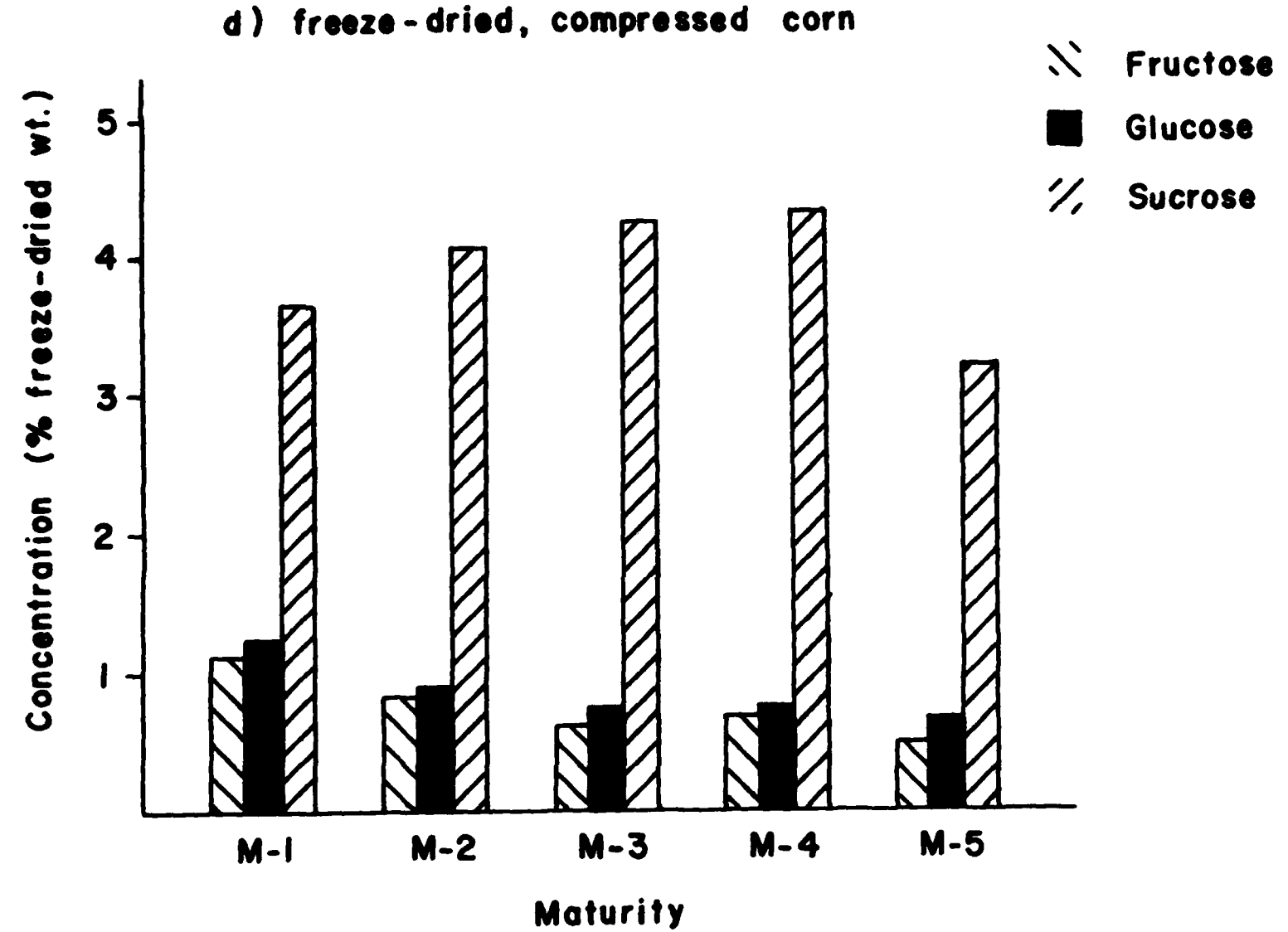


TABLE 1. MOISTURE CONTENT OF CORN SAMPLES (WT %)

	M-1	M-2	M-3	M-4	M-5
Frozen Corn	77.6	72.3	72.8	72.0	69.1
Steam Blanched, Sulfited and Frozen Corn	72.2	73.0	72.1	71.8	69.9
Freeze-Dried Corn	1.6	1.3	1.3	1.7	1.7
Freeze-Dried, Compressed Corn	6.7	7.1	7.1	6.8	6.6

TABLE 2. ALCOHOL INSOLUBLE SOLIDS OF CORN SAMPLES (WT %)

	M-1	M-2	M-3	M-4	M-5
Frozen Corn	22.9	21.9	22.5	21.8	25.2
Steam Blanched, Sulfited and Frozen Corn	22.1	21.4	23.3	23.2	24.8
Freeze-Dried Corn	80.3	80.9	81.6	81.9	82.5
Freeze-Dried, Compressed Corn	74.4	75.3	76.6	76.5	77.3